Amdt. dated December 17, 2008

Reply to Office Action of February 20, 2008

Amendments to the Claims:

1. (canceled)

- (currently amended) A method of assaying a Brassica plant for imidazolinone herbicide tolerance conferred by the PM1 mutation of the B. napus AHASI gene, the method comprising the steps of:
 - a) isolating genomic DNA from the plant;
 - b) selectively amplifying an AHASI gene from the genomic DNA using an AHASI forward primer having a the sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:9 and an AHASI reverse primer in a first amplification step, thereby producing an AHASI reaction mixture;
 - removing the AHASI primers from the AHASI reaction mixture to produce a purified AHASI reaction mixture;
 - d) in a second amplification step, further amplifying a portion of the amplified AHASI gene containing the site of the PM1 mutation, by combining the purified AHASI reaction mixture with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer bind to sites nested within the amplified portion of the AHASI gene;
 - e) denaturing the product of the second amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions; and
 - f) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded polynucleotide conformers in a substrate.

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- (currently amended) A method of assaying a Brassica plant for imidazolinone herbicide tolerance conferred by the PM1 mutation of the B. napus AHASI gene, the method comprising the steps of:
 - a) isolating genomic DNA from the plant;
 - selectively amplifying an AHASI gene from the genomic DNA using an AHASI forward primer and an AHASI reverse primer having a the sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:10 in a first amplification step, thereby producing an AHASI reaction mixture;
 - c) removing the AHASI primers from the AHASI reaction mixture to produce a purified AHASI reaction mixture;
 - d) in a second amplification step, further amplifying a portion of the amplified AHASI gene containing the site of the PMI mutation, by combining the purified AHASI reaction mixture with a PMI forward primer and a PMI reverse primer, wherein the PMI forward primer and the PMI reverse primer bind to sites nested within the amplified portion of the AHASI gene;
 - e) denaturing the product of the second amplification step to produce single stranded polvnucleotides that are allowed to adopt unique conformations by intramolecular interactions; and
 - f) detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded polynucleotide conformers in a substrate.
- 4. (previously presented) The method of claim 2 or 3, wherein the PM1 forward primer has a sequence as set forth in nucleotides 1 to 21 of SEQ ID NO:11.
- 5. (previously presented) The method of claim 2 or 3, wherein the PM1 reverse primer has a sequence as set forth in nucleotides 1 to 21 of SEQ ID NO:12.

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 (previously presented) The method of claim 2 or 3, wherein step (d) includes incorporating a label into the amplified portion of the AHASI gene.

- (original) The method of claim 6, wherein the label is selected from the group consisting of a radioactive label, a fluorescent label, a luminescent label, and a paramagmetic label.
- 8. (previously presented) The method of claim 2 or 3, wherein the substrate is selected from the group consisting of polyacrylamide, linear polyacrylamide, poly(N,N-dimethylacrylamide), hydroxyalkyl cellulose, polyoxyethylene, F127, agarose, diethylaminoethyl cellulose, sepharose, POP4, and POP6.
- 9. (previously presented) The method of claim 2 or 3, wherein the detection method is selected from the group consisting of electrophoresis and chromatography.
- 10. (previously presented) The method of claim 2 or 3, further comprising the step of detecting the presence or absence of PM2-mediated imidazolinone resistance in the plant.
- 11. (canceled)
- 12. (currently amended) A method for assaying a Brassica plant for imidazolinone herbicide tolerance conferred by the PM2 mutation of the B. napus AHAS3 gene, the method comprising the steps of:
 - a) isolating genomic DNA from the plant;
 - b) selectively amplifying the AHAS3 gene from the genomic DNA using an AHAS3 forward primer having a the sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:13 and an AHAS3 reverse primer in a first amplification step to produce an AHAS3 reaction mixture;
 - removing the AHAS3 primers from the AHAS3 reaction mixture to produce a purified AHAS3 reaction mixture;

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- d) in a second amplification step, further amplifying the amplified AHAS3 gene, by combining a first aliquot of the purified AHAS3 reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for a wild type allele of the PM2 region at position 1712 of the AHAS3 gene as depicted in SEQ ID NOs:5 and 8;
- e) in a third amplification step further amplifying the amplified AHAS3 gene, by combining a second aliquot of the purified AHAS3 reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for the PM2 mutation; and
- f) analyzing the amplified first and second aliquots for the presence or absence of the PM2 mutation.
- 13. (currently amended) A method for assaying a Brassica plant for imidazolinone herbicide tolerance conferred by the PM2 mutation of the B. napus AHAS3 gene, the method comprising the steps of:
 - a) isolating genomic DNA from the plant;
 - selectively amplifying the AHAS3 gene from the genomic DNA using an AHAS3 forward primer and an AHAS3 reverse primer having a the sequence as set forth in nucleotides 1 to 23 of SEQ ID NO:14 in a first amplification step to produce an AHAS3 reaction mixture;
 - c) removing the AHAS3 primers from the AHAS3 reaction mixture to produce a purified AHAS3 reaction mixture;
 - d) in a second amplification step, further amplifying the amplified AHAS3 gene, by combining a first aliquot of the purified AHAS3 reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for a wild type allele of the PM2 region at position 1712 of the AHAS3 gene as depicted in SEO ID NOs:5 and 8:
 - e) in a third amplification step further amplifying the amplified AHAS3 gene, by combining a second aliquot of the purified AHAS3 reaction mixture with a

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PM2 region forward primer, a PM2 region reverse primer, and a primer selective for the PM2 mutation; and

- f) analyzing the amplified first and second aliquots for the presence or absence of the PM2 mutation.
- 14. (previously presented) The method of claim 12 or 13, wherein the PM2 region forward primer has a sequence as set forth in nucleotides 1 to 19 of SEQ ID NO:15.
- 15. (previously presented) The method of claim 12 or 13, wherein the PM2 region reverse primer has a sequence as set forth in nucleotides 1 to 19 of SEQ ID NO:16.
- 16. (previously presented) The method of claim 12 or 13, wherein the wild type allele of the PM2 region at position 1712 has a sequence as set forth in nucleotides 1 to 18 of SEQ ID NO:17.
- 17. (previously presented) The method of claim 12 or 13, wherein the primer selective for the PM2 mutation has a sequence as set forth in nucleotides 1 to 20 of SEO ID NO:18.
- 18. (previously presented) The method of claim 12 or 13, wherein steps (d) and (e) include incorporating a label into the amplified portion of the *AHAS3* gene.
- 19. (original) The method of claim 18, wherein the label is selected from the group consisting of a radioactive label, a fluorescent label, a luminescent label, and a paramagnetic label.
- 20. (previously presented) The method of claim 12 or 13, wherein the analyzing step employs a method selected from the group consisting of electrophoresis and chromatography.

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- 21. (previously presented) The method of claim 12 or 13, further comprising the steps of:
 - g) selectively amplifying an AHASI gene from the genomic DNA using an AHASI forward primer and an AHASI reverse primer in a fourth amplification step;
 - h) removing the AHASI primers from the product of step g);
 - i) in a fifth amplification step, further amplifying a portion of the amplified AHAS1 gene containing the site of the PM1 mutation, by combining the product of step h) with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer bind to sites nested within the amplified portion of the AHAS1 gene;
 - j) denaturing the product of the fifth amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions; and
 - detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded conformer polynucleotides in a substrate.
- 22. (canceled)
- (canceled)
- 24. (currently amended) A method of marker assisted breeding of plants of Brassica species using a PM1 mutation of the B. napus AHASI gene as a marker, the method comprising the steps of:
 - a) isolating genomic DNA from a Brassica plant;
 - b) selectively amplifying an AHASI gene from the genomic DNA using an AHASI forward primer having a the sequence as set forth in nucleotides 1 to 22 of SEQ ID NO:9 and an AHASI reverse primer in a first amplification step, thereby producing an AHASI reaction mixture;

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- removing the AHASI primers from the AHASI reaction mixture to produce a purified AHASI reaction mixture;
- d) in a second amplification step, further amplifying a portion of the amplified AHASI gene containing the site of the PM1 mutation, by combining the purified AHASI reaction mixture with a PM1 forward primer and a PM1 reverse primer, wherein the PM1 forward primer and the PM1 reverse primer bind to sites nested within the amplified portion of the AHASI gene;
- e) denaturing the product of the second amplification step to produce single stranded polynucleotides that are allowed to adopt unique conformations by intramolecular interactions;
- detecting the presence or absence of the PM1 mutation on the basis of the mobility of said single stranded polynucleotide conformers in a substrate; and
- g) selecting said plant as a parent for further breeding if the PM1 mutation is present.
- 25. (currently amended) A method of marker assisted breeding of plants of Brassica species using a PM2 mutation of the B. napus AHAS3 gene as a marker, the method comprising the steps of:
 - a) isolating genomic DNA from the plant;
 - b) selectively amplifying the AHAS3 gene from the genomic DNA using an AHAS3 forward primer and an AHAS3 reverse primer having a the sequence as set forth in nucleotides 1 to 22 23 of SEQ ID NO:40 14 in a first amplification step to produce an AHAS3 reaction mixture:
 - removing the AHAS3 primers from the AHAS3 reaction mixture to produce a purified AHAS3 reaction mixture;
 - d) in a second amplification step, further amplifying the amplified AHAS3 gene, by combining a first aliquot of the purified AHAS3 reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for a wild type allele of the PM2 region at position 1712 of the AHAS3 gene as depicted in SEQ ID NOs:5 and 8;

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- e) in a third amplification step further amplifying the amplified AHAS3 gene, by combining a second aliquot of the purified AHAS3 reaction mixture with a PM2 region forward primer, a PM2 region reverse primer, and a primer selective for the PM2 mutation;
- f) analyzing the amplified first and second aliquots for the presence or absence of the PM2 mutation; and
- selecting said plant as a parent for further breeding if the PM2 mutation is present.